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13. ABSTRACT ***(Maximum 200 Words)***
The goal of this project is to analyze the metastasis of mammary tumors in transgenic mice. This will make use of fluorescently labeled tumor cells to allow in vivo visualization of the tumor cells in the primary tumor as well as in target organs. For the first year of this project we have generated 3 transgenic lines carrying the MMTV-GFP expression construct. We have evaluated GFP fluorescence in one of these lines (founder 25) and are in the process of testing two others. Heterozygotes derived from founder 25 show GFP fluorescence in mammary ducts and are now being crossed with transgenic mice carrying the HER2/neu or polyoma middle T transgene.

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FOREWORD

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PI - Signature Date

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Introduction

The major life threatening aspect of most cancers involves the metastatic spread of cancer. However, methods for dissecting the process of metastasis need further development in order to be able to determine the functions of specific gene products in metastasis. Transgenic mice expressing or lacking specific cancer related genes have provided a powerful method for evaluating mechanisms of tumor formation. This project will develop new techniques for more detailed dissection of the roles of such gene products in the metastatic process, using GFP (green fluorescent protein)-expressing transgenic mice. In the first technical objective, transgenic mice expressing GFP in the mammary gland has been generated. We will first evaluate an expression construct in which the enhanced GFP cDNA with mammalian codon sequences is driven by the full length MMTV promoter and ends with the SV40 intron and polyadenylation sequence. Transgenic mice which have the highest level of GFP expression in the mammary gland and greatest tissue specificity of expression will be chosen for further work. In the second technical objective, these mice will then be used together with image analysis techniques that we have developed in order to dissect the process of metastasis in more detail. By crossing the GFP-mice with mice containing specific mammary tissue-targeted proteins such as HER2/neu, the contributions of specific oncogenes to the metastatic cascade will be determined. The properties of tumor cells at the border of the tumor, the density of tumor cells in the blood, the kinetics of invasion of target organs, and the distribution of individual tumor cells and metastases in target organs such as the lung can be evaluated using such mice. In the third technical objective, the contribution of other proteins to the various stages of metastasis, such as angiogenesis factors, proteases, cytoskeletal proteins, and adhesion proteins will be determined. In this first annual report, we describe nearly completing the first technical objective, and beginning work on the second objective.

Body

The technical objectives of this project are:

Technical Objective 1: To generate transgenic mice carrying constructs targeting GFP to the mammary gland and to evaluate the specificity of GFP expression.

Technical Objective 2: To cross mice expressing GFP in the mammary gland with mice expressing HER2/neu under the control of the MMTV promoter and utilize the GFP expression to evaluate the metastatic properties of the tumors that develop in these mice.

Technical Objective 3: To evaluate the effects of other transgenes or knockouts on the rate limiting step of metastasis of HER2/neu tumors.

The statement of work is:

STATEMENT OF WORK

Task 1: To generate transgenic mice expressing green fluorescent protein in the mammary gland (months 1 - 12).

- develop expression constructs for mammary targeted expression of GFP (months 1 -3)
- generate founder mice with the aid of the Transgenic Core Facility (months 3 -6)

- identify founders that stably transmit the GFP gene, have high mammary specific expression of GFP (months 6 - 12). This may require testing up to 6 founders with crosses for 2 generations to test transmission and tissue specificity. Up to 240 newborn mice or 72 transgenics may be evaluated.

Task 2. To cross the GFP transgenic mice with HER2/neu mice and evaluate the metastatic cascade for the tumors that develop (months 12 - 24).

- Use at least one test cross to refine assays for analysis of tumor cells in blood and injection of tumor cells for analysis of seeding in lungs (months 13 - 19).Per cross, if two heterozygote trangenics are crossed, to acquire around 20 double transgenics will require around 80 progeny. 10 single transgenic progeny will also be kept to as controls for background effects, although both transgenics are in the FvB background.
- perform the cross and allow tumors to develop as tumors reach .5 - 1 cm, sacrifice mice and analyze the distribution of tumor cells (months 15 - 24).

Task 3. To cross GFP HER2/neu mice with mice defective in matrilysin or gelsolin and evaluate the contributions of these molecules to the various metastatic steps (months 24 -36).

- perform the crosses and allow tumors to develop (months 24 - 30). Per cross, if two heterozygote transgenics are crossed, to acquire around 20 double transgenics will require around 80 progeny. 10 single transgenic progeny will also be kept to as controls for background effects.
- as tumors reach .5 - 1 cm, sacrifice mice and analyze the distribution of tumor cells (months 30 - 36).

Progress for Year 1

We have nearly fulfilled Task 1 as follows.

- develop expression constructs for mammary targeted expression of GFP (months 1 -3)

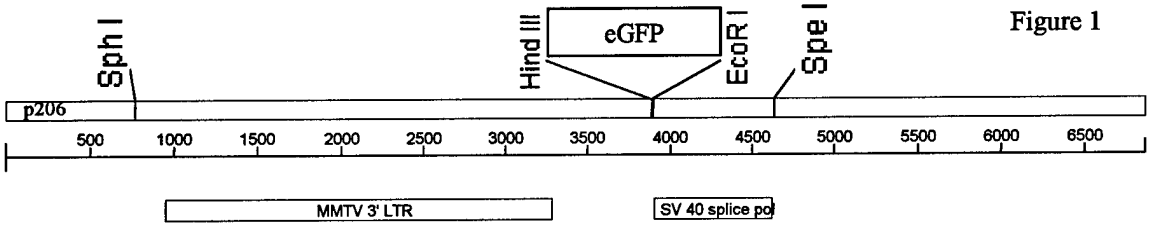
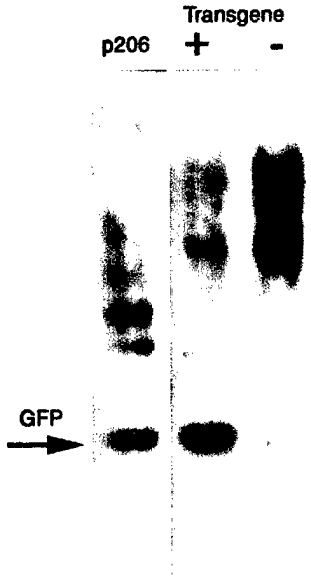


Figure 1

We subcloned the coding sequence of the enhanced GFP gene from pEGFPN1 into p206 as follow (Figure 1). EGFP N1 was digested with Not I and p206 was digested with Eco RI. Both digested plasmids were then blunted with Klenow fragment and then digested with HindIII. The eGFPN1 fragment was then ligated into the digested p206, resulting in the construct shown in Figure 1. the MMTV LTR provides specific transcription in the mammary gland while the SV40 splicing and polyadenylation fragment enhances export and translation. The construct was

then digested with SphI and SpeI and the expression construct was used to generate potential founder mice in the Transgenic Mouse Facility at Einstein.

Figure 2

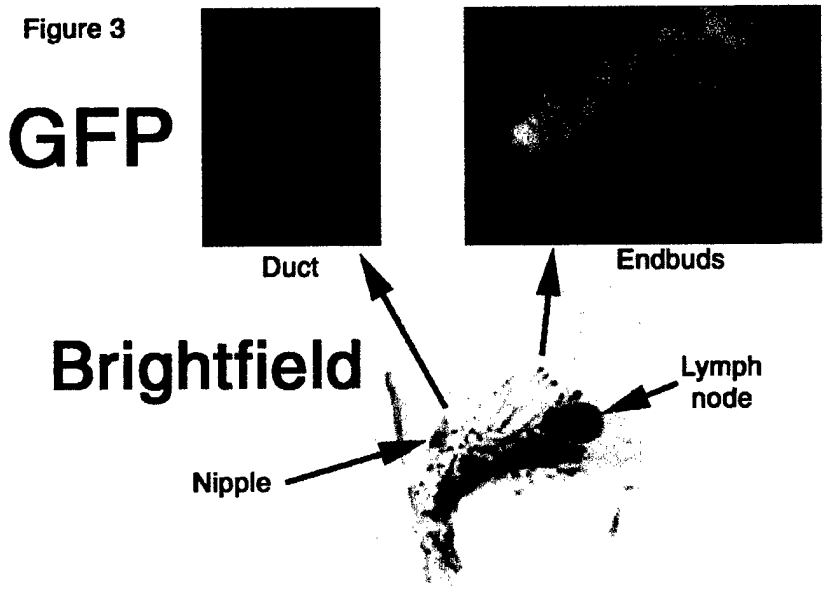


- generate founder mice with the aid of the Transgenic Core Facility (months 3 - 6)

The transgenic facility provided us with 60 potential founders. We first attempted using PCR to identify transgenic animals but found that more consistent results could be obtained using Southern blotting. Using Southern blotting we identified 4 transgenic mice (Figure 2). Crossing them with nontransgenic Fvb mice, we found that 2 founders (25 and 57) transmitted the transgene efficiently, one transmitted at low efficiency, and 1 has been unable to mate.

- identify founders that stably transmit the GFP gene, have high mammary specific expression of GFP (months 6 - 12). This may require testing up to 6 founders with crosses for 2 generations to test transmission and tissue specificity. Up to 240 newborn mice or 72 transgenics may be evaluated.

Figure 3



Founder 25 has been studied most thoroughly at this point. Analysis of the mammary glands using the fluorescence microscope demonstrates that the ducts and potentially alveolar structures are detectable in transgenic mice (Figure 3) but not in control nontransgenics. In Figure 3, the brightfield image shows a typical mammary gland stained to show ductal and alveolar structures. The GFP fluorescent images on top are from an unstained mammary gland, showing structures corresponding to the brightfield image. Although we are continuing to test the fluorescence levels of heterozygous animals to determine the relative intensity increase

as well as the fluorescence present in the 57 line, we are beginning crosses with transgenic animals expressing HER2/neu and polyoma middle T antigen in the mammary gland. The polyoma transgenics generate tumors much more rapidly than the HER2/neu animals and will provide us with more opportunities to refine our methods of imaging tumors in transgenic mice.

Key Research Accomplishments

- Generation of transgenic mice expressing GFP in the mammary gland under the control of the MMTV LTR.

Reportable Outcomes

None at this time.

Conclusions

We have fulfilled Task 1 of the project and are on schedule. We have generated transgenic mice expressing GFP in the mammary gland and will next evaluate the opportunity to image tumors using fluorescence microscopy in vivo.